Metabolism of 2-*sec*-Butylphenyl *N*-Methylcarbamate (Bassa[®], BPMC) in Rice Plants and Its Degradation in Soils

Kunihiko OGAWA,* Masataka TSUDA,** Fumio YAMAUCHI,*
Isamu YAMAGUCHI and Tomomasa MISATO

The Institute of Physical and Chemical Research, Wako, Saitama 351, Japan

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Absorption, translocation, and metabolism of methylcarbamate insecticide, 2-*sec*-butylphenyl *N*-methylcarbamate (Bassa[®], BPMC), in rice plants and its degradation in soils were studied by using ¹⁴C-Bassa labeled at 1-methyl of *sec*-butyl group of ring substituted side chain.

On rice plants, ¹⁴C-Bassa was absorbed from the root and leaves, and then translocated to an upper part and an apical part of a leaf from the treated position. Most of radio-carbon treated on leaves or ear of rice plant, however, was rapidly lost by evaporation from the surface. In plants, Bassa was oxidized at *sec*-butyl, *N*-methyl group, and 4-position of aromatic ring, and then these oxidized metabolites were conjugated with β -glucose or others. The main metabolite was 2-(1-methyl 2-hydroxypropyl)phenyl *N*-methylcarbamate.

In soils, degradation of ¹⁴C-Bassa resulted in the oxidation of *sec*-butyl group, *N*-demethylation, and hydrolysis of ester linkage and gave rise to 2-(1-acetyylethyl)phenyl *N*-methylcarbamate, 2-*sec*-butylphenylcarbamate, and 2-*sec*-butylphenol, respectively. A sixth to 1/3 of radioactivity remained unextractable as soil-bound residues 30 days after the treatment.

All of these metabolites were assumed to be less toxic than the parent compound based on the anticholinesterase activity except 2-*sec*-butyl 4-hydroxyphenyl *N*-methylcarbamate, which, however, was found only as a conjugated form in rice plants.

INTRODUCTION

2-*sec*-Butylphenyl *N*-methylcarbamate (Bassa[®], BPMC) is an effective insecticide for the control of planthoppers and leafhoppers of rice plants, and is used in large quantities among methylcarbamate insecticides in Japan.

It is important for public health and environmental safety to investigate the fate of pesticides in crops and animals, degrada-

tion in soils and water, and photodegradation. Hitherto, the metabolism of *N*-methylcarbamate pesticides has been reviewed on animals,¹⁾ insects and plants,²⁾ and soils.³⁾ In the case of Bassa, however, only the metabolism by soil fungi has been reported.⁴⁾ Its main metabolite was a compound hydroxylated at 2-position of *sec*-butyl group, and others were phenolic compounds, alcoholic, ketonic, and carboxylic compounds oxidized at *sec*-butyl group, and also compounds hydroxylated and demethylated at *N*-methyl group.

This paper deals with the absorption, translocation, and metabolism of Bassa in rice plants, its degradation in soils, and estimation for biological activity of these metabolites.

*,** Present address: * Analytical Department, Systems Engineering Laboratory, and ** Agricultural Chemicals Department, Biosciences Laboratory, Central Research Laboratories, Mitsubishi Chemical Industries Ltd., Midori-ku, Yokohama 227, Japan.

MATERIALS AND METHODS

1. Chemicals

^{14}C -Bassa labeled at 1-methyl of *sec*-butyl group (4.89 mCi/mmol, radiochemical purity more than 99.9% by TLC) was obtained from Daiichi Pure Chemicals Co. Ltd., Japan. Unlabeled carbamate and its related compounds shown in Table 1 were synthesized, and their structures were confirmed by IR, NMR, and MS. β -Glucosidase (almonds) was obtained from Miles-Seravac Ltd., England. Dain (sticker) was obtained from Takeda Chemical Industries Ltd., Japan.

2. Radioanalysis and Autoradiography

Radioactivity was counted by liquid scintillation counter (LSC-652, Aloka Co. Ltd., Japan) using either of 15 ml of the following scintillator solutions; A: 4.0 g PPO in 1 l of toluene; B: 8.0 g PPO, 100 g naphthalene and 20 ml ethyleneglycol in 1 l of dioxane. Un-

extractable radioactivity in plants was counted after combustion treatment by an automatic sample oxidizer (Tri-carb, Packard Instrument Co. Inc., U.S.A.). Soil-bound residues were oxidized using CrO_3 and H_3PO_4 as described by Nömmik⁵⁾ prior to radioanalysis. Autoradiograms (ARG) of the developed TLC or plants were taken by exposing them to X-ray film (Fuji Kx Medical, Fuji Photo Film Co. Ltd., Japan) for several days at low temperature (4°C).

3. Thin Layer Chromatography

TLC plate precoated with 0.25 mm thick silicagel G (E. Merck, W. Germany) and sandwich type developer (Yamato Scientific Co. Ltd., Japan) were used for TLC. Two-dimensional development was adopted for separation and co-chromatographic identification of metabolites. Ether-*n*-hexane (4:1, v/v) was used for the first development system, and chloroform-acetonitrile (4:1, v/v)

Table 1 A list of Bassa and its related compounds and their R_f values on TLC.

Abbreviation	Chemical structure	R_1	R_2	R_3	R_f values of TLC*	
					I	II
BPMC		$-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$	$-\text{H}$	$-\text{H}$	0.69	0.82
B-1-OH		$-\text{C}(\text{CH}_3)(\text{OH})\text{CH}_2\text{CH}_3$	$-\text{H}$	$-\text{H}$	0.36	0.44
B-2-OH		$-\text{CH}(\text{CH}_3)\text{CH}(\text{OH})\text{CH}_3$	$-\text{H}$	$-\text{H}$	0.21	0.35
B-3-OH		$-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{OH}$	$-\text{H}$	$-\text{H}$	0.21	0.30
B-1- CH_2OH		$-\text{CH}(\text{CH}_2\text{OH})\text{CH}_2\text{CH}_3$	$-\text{H}$	$-\text{H}$	0.21	0.30
B-4-OH		$-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$	$-\text{OH}$	$-\text{H}$	0.44	0.46
B-5-OH		$-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$	$-\text{H}$	$-\text{OH}$	0.39	0.35
B-2-CO		$-\text{CH}(\text{CH}_3)\text{COCH}_3$	$-\text{H}$	$-\text{H}$	0.44	0.69
B-1-COOH		$-\text{CH}(\text{COOH})\text{CH}_2\text{CH}_3$	$-\text{H}$	$-\text{H}$	0.07	0.07
B-2-COOH		$-\text{CH}(\text{CH}_3)\text{CH}_2\text{COOH}$	$-\text{H}$	$-\text{H}$	0.13	0.12
OSBP		$-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$	$-\text{H}$	$-\text{H}$	0.85	0.84
P-1-OH		$-\text{C}(\text{CH}_3)(\text{OH})\text{CH}_2\text{CH}_3$	$-\text{H}$	$-\text{H}$	0.80	0.79
P-2-OH		$-\text{CH}(\text{CH}_3)\text{CH}(\text{OH})\text{CH}_3$	$-\text{H}$	$-\text{H}$	0.64	0.64
P-3-OH		$-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{OH}$	$-\text{H}$	$-\text{H}$	0.49	0.41
P-1- CH_2OH		$-\text{CH}(\text{CH}_2\text{OH})\text{CH}_2\text{CH}_3$	$-\text{H}$	$-\text{H}$	0.59	0.52
P-4-OH		$-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$	$-\text{OH}$	$-\text{H}$	0.68	0.53
P-5-OH		$-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$	$-\text{H}$	$-\text{OH}$	0.57	0.33
P-2-CO		$-\text{CH}(\text{CH}_3)\text{COCH}_3$	$-\text{H}$	$-\text{H}$	0.72	0.74
B-N-OH		$-\text{CON}(\text{OH})\text{CH}_3$	$-\text{H}$	$-\text{H}$	0.67	0.68
B-N- CH_2OH		$-\text{CONHCH}_2\text{OH}$	$-\text{H}$	$-\text{H}$	0.42	0.40
B-NH ₂		$-\text{CONH}_2$	$-\text{H}$	$-\text{H}$	0.60	0.71

* solvent system; I=ether-*n*-hexane (4:1, v/v),
II=chloroform-acetonitrile (4:1, v/v).

for the second one. *R_f* values of authentic samples were shown in Table 1. Because B-1-CH₂OH and B-3-OH were not separated each other with these solvent systems on TLC, they were determined as their phenolic compounds, P-1-CH₂OH and P-3-OH, after hydrolysis with methanolic 0.1N sodium hydroxide solution for 30 min. Metabolites and authentic samples developed on TLC plates were detected as yellow to red spots by spraying 10% sodium hydroxide aqueous solution and thereafter methanolic 0.05% 4-nitrobenzenediazoniumfluoroborate solution.

4. Absorption and Translocation of ¹⁴C-Bassa by Rice Plants via Roots

Rice plants (Jukkoku) were cultured for 14 days in plastic flower pots containing vermiculite. The plants at 3 to 4 leaf stage were removed from the pots and their roots were washed carefully with water. The roots of two rice seedlings were dipped in an aqueous solution (20 ml) containing 80 μg (3.0 μCi) of ¹⁴C-Bassa in a greenhouse. One, 6, 24, and 48 hrs after dipping, absorption and translocation of ¹⁴C-Bassa were determined from its radioactivity in rice plants and cultivating solution by ARG and radioanalysis with liquid scintillation counter (LSC).

5. Absorption and Translocation of ¹⁴C-Bassa by Rice Plants through Leaves and by Culm Injection Method

Rice plants (Sasanishiki) were cultured in 1/5000 are-sized Wagner's pots until tillering stage. They were treated, as uniformly as possible, on the upper surface of three leaves from the top with 100 μl of solution containing 220 μg (5.1 μCi) of Bassa and 2 mg of Dain in methanol-water (1:3) per plant, or slowly injected to culm with 50 μl of ¹⁴C-Bassa (5.1 μCi) in methanol-water (1:1). The applied plants were kept in a greenhouse and supplied with water when necessary. After 1, 3, 9, 27, and 62 days (the harvest time), the rice plants were cut on soil surface in order to determine absorption and translocation by ARG.

6. Metabolism in Rice Plants

The rice plants were cultured in Wagner's

pots until tillering and milk-ripening stage. At milk-ripening stage, a plant was treated, as uniformly as possible, on the surface of two leaves from the top and the husk of ears with ¹⁴C-Bassa solution. Periodically the rice plants were cut on the soil surface, and as for the rice plant at harvest time they were divided into straw, husk, grain, and root. After rinsed with ether to remove the surface residue, these specimens were cut into small pieces and extracted with acetone, chloroform, and water, using Abdel-Wahab's method,⁶⁾ and fractionated as follows; the surface wash, the acetone-chloroform extracts, the water extracts, and the unextractable residues. The water extracts containing conjugated metabolites were extracted with ether after hydrolysis by β-glucosidase, and residual solution was further hydrolyzed with 2N HCl (80°C, 3 hrs). The metabolites and hydrolyzed compounds were detected by ARG after TLC, and were identified by co-chromatography with authentic samples. Their radioactivities were determined by LSC.

7. Degradation in Soils

Two types of paddy field soils (Tochigi; volcanic ash soil, clay loam, clay 34%, total carbon 9.2%, pH 6.4, and Kochi; mineral soil, loam, clay 21%, total carbon 2.5%, cation exchange capacity 10.1 meq/100 g dry soil) and two types of upland field soils (Tochigi; the same values with those of paddy field soil, and its maximum water capacity 107%, and Kochi; mineral soil, loam, clay 34%, total carbon 1.2%, 17.8 meq/100 g dry soil, maximum water capacity 77%) were employed for experiment. Each soil (10 g of dry weight basis) was added in Erlenmeyer's flask with an equipment for trapping ¹⁴CO₂. The paddy field soils were flooded, and the upland field soils were added with water to adjust 60% of its maximum water capacity. After incubated at 30°C for one week, the solution of ¹⁴C-Bassa in methanol-water (1:9) was added to the soils at the concentration of 20 ppm on a dried soil weight basis, and thoroughly mixed before incubation at 30°C. Two, 6, 14 and 30 days after incubation, the paddy field soils were extracted with

30 ml of water, 50 ml of acetone, and 50 ml of ether, and the upland field soils were extracted in reverse order. Degradation products in the ether extracts were detected by ARG after TLC, identified by co-chromatography, and their amounts were determined by LSC. The generated $^{14}\text{CO}_2$ was trapped in ethanolamine-methylcellosolve (1:3) and was determined by LSC.

8. Anticholinesterase Activity

The biological activities of Bassa metabolites were estimated by their inhibitory activities determined by Ellman's method⁷⁾ on cholinesterase of green rice leafhopper homogenates, horse serum pseudo cholinesterase (Sigma Chemical Co., U.S.A.) and human serum pseudo cholinesterase (Sigma Chemical Co., U.S.A.).

RESULTS

1. Absorption and Translocation of Bassa in Rice Plants

Six, 24 and 48 hrs after root dipping, ARG of the treated plant was taken. As shown

Fig. 1 Autoradiogram of rice plant after root dipping in aqueous solution of ^{14}C -Bassa for 24 hrs.

Fig. 2 Absorption of radiocarbon by rice plants during root dipping in aqueous solution of ^{14}C -Bassa.

distribution of radioactivity: $\square$ acetone-chloroform extracts, $\triangle$ water extracts, $\times$ unextractable residues, $\bullet$ cultivating solution.

in Fig. 1, Bassa in cultivating solution was absorbed from the root of rice seedling and translocated to all part of the plant, and more dominantly in an apical or subapical part of a leaf. Fig. 2 shows distribution of radioactivity in the plants and solution during root dipping treatment. Radioactivity in plants increased rapidly until 6 hrs after dipping, but slightly increased subsequently. Most of radioactivity in plants was extracted with acetone and chloroform, and about 90% of the extracted radioactivity was attributed to intact ^{14}C -Bassa even 24 hrs after treatment.

When ^{14}C -Bassa were applied on leaves of rice plants at the tillering stage, the radioactivity decreased from a basal part of a treated leaf and concentrated at its apical part (Fig. 3). On culm injection, it translocated with transpiration stream into the growing leaves and an apical part of a leaf. But radioactivity translocated little to lower part, and at the harvest time, it was not observed in a flag leaf and an ear (Fig. 4).

2. Metabolism in Rice Plants

The distribution of radioactivity after an application of ^{14}C -Bassa on rice plant of tillering and milk-ripening stage is shown in

Fig. 3 Autoradiogram (right) of a rice plant smeared on leaves with ^{14}C -Bassa. Treated regions are indicated by arrows. A: 3 days after treatment, B: 62 days after treatment.

Fig. 4 Autoradiogram (right) of a rice plant injected in culm with ^{14}C -Bassa. Injected parts are indicated by arrows. A: 3 days after treatment, B: 62 days after treatment.

Fig. 5 Distribution of radioactivity in rice plants after tillering stage application (A) and milk-ripening stage application (B) of ^{14}C -Bassa on leaves and ear. ● total, ○ surface wash, □ acetone-chloroform extracts, △ water extracts, × unextractable residues.

Fig. 6 Thin-layer chromatographic separation of ^{14}C -Bassa and its metabolites in rice plant. U; unknown metabolite.

Fig. 5. Ninety-five per cent and 87% of radioactivity were not recovered 9 days after the application at tillering and milk-ripening stage, respectively. These were supposed to be lost by volatilization from plants. The remainder was absorbed into plants and metabolized.

The radioactive metabolites in the three fractions except unextractable residues were detected by ARG after TLC as shown in Fig. 6. Among metabolites, fifteen compounds were identified by co-chromatography with authentic samples. Main metabolites, B-2-OH, B-3-OH, B-4-OH, B-1-CH₂OH, B-N-CH₂OH, OSBP, and P-2-OH, were identified by GC-MS (M-52, Hitachi Co. Ltd., Japan) directly or after trifluoroacetylation.

Table 2 shows the amount of main metabolites in rice plants applied at tillering and milk-ripening stage. In surface wash, the main metabolites were B-2-OH and in acetone-chloroform extracts, B-1-OH and B-2-OH. In water extracts, metabolites were supposed to exist as conjugates, since β -glucosidase hydrolysis of the extracts afforded ether soluble B-2-OH, B-4-OH, and B-N-CH₂OH. After hydrolysis of the extracts by HCl, B-NH₂ and P-2-OH were also recognized. As the plant of tillering stage was quickly growing, the applied Bassa was rapidly metabolized, so that at the harvest time only a few amount of Bassa and other metabolites remained. But in case of milk-ripening stage application, the metabolism was comparatively slower, so that residual amount of Bassa and its metabolites was

Table 2 Metabolites of ^{14}C -Bassa in rice plants.

Metabolites	Radiocarbon recovered as indicated products, %				
	Tillering stage application	Milk-ripening stage application			
		3 days**	22 days**		
			Straw	Husk	Grain
Surface wash					
BPMC	0.02	0.54	0.04	0.04	—
B-2-OH	0.15	0.02	0.01	—	—
Others***	0.15	0.09	0.01	0.02	—
Acetone-chloroform extracts					
BPMC	0.08	7.30	0.78	1.46	0.37
B-1-OH	0.03	0.18	0.12	0.04	0.02
B-2-OH	0.34	0.84	0.44	0.14	0.07
B-N-CH ₂ OH	—	0.26	0.05	0.07	0.02
Others***	0.28	0.58	0.22	0.11	0.03
Water extracts					
(β -Glucosidase-hydrolyzed metabolites)					
B-2-OH	0.06	0.10	0.06	0.01	0.03
B-4-OH	—	0.19	0.02	—	—
B-N-CH ₂ OH	0.02	0.08	0.03	—	—
Others***	0.04	0.23	0.12	0.03	0.04
(HCl-hydrolyzed metabolites)					
B-2-OH	0.03	0.20	0.19	0.10	0.31
B-NH ₂	0.02	0.06	0.04	0.02	0.06
P-2-OH	0.04	0.41	0.05	0.04	0.13
Others***	0.05	0.20	0.13	0.05	0.20
Unextractable residues (Plant pulp)	1.70	4.30	2.47	0.54	0.36
Total	3.01	15.58	4.78	2.67	1.64

* Total % in husk, grain, and root were less than 0.01% to 0.03% of radiocarbon treated.

** Total % in root was less than 0.01% of radiocarbon treated.

*** Other identified metabolites and unknowns.

more than of tillering stage application. Some amount of Bassa applied on husk of an ear was observed to translocate into grains, and metabolites in the grains were largely extractable with water. After hydrolysis of the extracts by HCl, B-2-OH was detected as a main metabolite.

3. Degradation in Soils

Degradation products in soils were separated by TLC after ether extraction and 22 radioactive metabolites were detected by ARG. The main degradation products were identified by co-chromatography on TLC and by MS to be B-2-CO, B-NH₂ and OSBP,

which were in good accordance with the metabolites found in rice plants. Table 3 shows the amount of main degradation products. On Tochigi soils OSBP was rather predominant, while on Kochi soils B-NH₂ and B-2-CO were observed significantly.

As shown in Fig. 7, the decrease of ether extractable radioactivity and increase of unextractable radioactivity were more rapid in Tochigi soils than in Kochi soils. This reflects the maximum generation of $^{14}\text{CO}_2$ on Tochigi upland field soil and the minimum on Kochi upland field soil; one third of radioactivity initially added was found as $^{14}\text{CO}_2$ in the former, while only 1.0% was

Table 3 Bassa and its degradation products in soils.

Soil	Time (Days)	Radiocarbon recovered as indicated products, %							
		Paddy field soils				Upland field soils			
		BPMC	B-2-CO	B-NH ₂	OSBP	BPMC	B-2-CO	B-NH ₂	OSBP
Tochigi	6	52.8	0.01	0.01	0.16	55.3	0.19	0.70	1.21
	14	37.1	0.11	0.08	0.51	12.5	0.07	0.24	0.35
	30	8.7	0.03	0.03	0.39	3.32	0.05	0.05	0.11
Kochi	6	77.4	0.25	0.15	0.30	57.1	0.36	1.03	0.52
	14	68.3	0.27	0.23	0.54	40.7	0.30	0.37	0.32
	30	30.8	0.19	0.17	0.47	33.3	0.21	0.36	0.44

Fig. 7 Radioactivity extracted from paddy field soils (A) and upland field soils (B) after ¹⁴C-Bassa treatment.
● total, ○ ether extracts, △ water extracts, × ¹⁴C remaining in soil.
solid line; Tochigi soils, broken line; Kochi soils.

Table 4 Inhibition to cholinesterase (ChE) of green rice leafhoppers, horse serum pseudo ChE, and human serum pseudo ChE by Bassa metabolites and related compounds.

Compound	I ₅₀ (M × 10 ⁻⁵)		
	Green rice leafhopper ChE	Horse serum pseudo ChE	Human serum pseudo ChE
BPMC	0.16	13	3.6
B-1-OH	2.1	94	
B-2-OH	5.3	>100	
B-3-OH	3.4	>100	
B-1-CH ₂ OH	2.6	>100	
B-4-OH	0.59	2.6	0.53
B-5-OH	0.31	7.5	5.8
B-2-CO	3.4	55	
OSBP	>100		
P-2-OH	>100		
B-N-OH	46	5.8	0.50
B-N-CH ₂ OH	>100	79	
B-NH ₂	1.5	67	

found in the latter.

4. Anticholinesterase Activity

Table 4 shows the inhibition to cholinesterase of green rice leafhopper homogenates, to horse serum pseudo cholinesterase, and to human serum pseudo cholinesterase by Bassa, its metabolites and related compounds. To the cholinesterase of green rice leafhopper homogenates, all of the metabolites tested were less inhibitory than Bassa. But B-4-OH indicated high inhibitory activity on other two cholinesterases. Though B-N-OH and B-5-OH also indicated high activity, they were not detected among metabolites.

DISCUSSION

In rice plants, Bassa was absorbed easily

from root and 11% of radioactivity was detected in the plant 24 hrs after dipping, which paralleled with its decrease in aqueous solution. On the other hand, Bassa treated on leaves or in culm was rapidly lost from leaves. Twenty-four hours after application on the leaves or ear surface, 79% to 86% of radioactivity were lost by volatilization, as well as other *N*-methylcarbamates on plants.⁸⁾ The absorbed Bassa was translocated to growing leaves and an apical part of a leaf, and then it was metabolized by the hydroxylation and oxidation of *sec*-butyl group and aromatic ring, the hydrolysis of ester linkage, and the hydroxylation and *N*-demethylation of *N*-methyl group. Then these metabolites were conjugated with β -glucose or others.

It has been reported that the substituted side chain of methylcarbamate was susceptible to be hydroxylated at 1-carbon position,⁹⁾ but in the case of Bassa, main metabolite was B-2-OH, which was hydroxylated at 2-carbon position of *sec*-butyl group. It re-

mained as free compound mainly in straw but as conjugated in grain. And it was suggested to be converted to P-2-OH by hydrolysis and subsequently conjugated (Fig. 8).

N-demethyl derivatives of methylcarbamate were reported to be found in homogenate of housefly,¹⁰⁾ in soil fungi,⁴⁾ and in photodegradation products,¹¹⁾ but these compounds have not been detected in the metabolites of rice plants. In this concern, it is interesting that B-NH₂, *N*-demethyl compound of Bassa was detected in HCl hydrolysate of water extract from rice plant, though the results obtained did not indicate directly the existence of demethyl derivatives *in vivo*, because it has been reported that *N*-hydroxymethyl compounds were easily decomposed to *N*-demethyl compounds by acidic conditions.¹²⁾

Degradation of Bassa was more rapid in Tochigi soils than in Kochi ones, and under upland conditions than under flooded conditions. Degradation products in ether extracts were almost the same as metabolites in

Fig. 8 Proposed metabolic pathways for Bassa in rice plants and soils.
P; plants, S; soils.

plants though their subsequent conjugation did not occur in soils. Compared with the metabolism in rice plants, further oxidation or hydrolysis proceeded in soils, so that the main degradation products were B-2-CO, B-NH₂, and OSBP (Table 3). It has been reported that the amount of unextractable radioactivity in soils was proportional to organic matter content.¹³⁾ On Tochigi soils, containing a great deal of organic matter, a large amount of radioactivity was retained, and the decrease of ether extractable radioactivity was more rapid and the generation of ¹⁴CO₂ was also larger. The degradation products in soils were in good agreement with the metabolites by soil fungi as described by Suzuki and Takeda.⁴⁾

In general, metabolism of pesticides convert them to less active compounds, but it has been reported that some of the aromatic ring hydroxylated compounds of *N*-methylcarbamates were more inhibitory against cholinesterase than the parent compounds.^{8,14)} Therefore the toxicological estimation of metabolites is also important for public health and environmental safety. The biological activity of Bassa metabolites was estimated by the inhibitory activity on 3 kinds of cholinesterases different from that described by Suzuki.¹⁵⁾ Inhibitory activities of all metabolites tested were lower than that of Bassa to cholinesterase of green rice leafhoppers, but B-4-OH, B-5-OH and B-N-OH indicated marked inhibition to horse serum pseudo cholinesterase or to human serum pseudo cholinesterase. Among them, however, only B-4-OH was detected in plants, and almost all of it was suggested to exist as conjugated form. But the biological significance of such conjugated metabolites has not yet been determined from the standpoint of inhibitory activity to cholinesterase.

Based on all of the results described above, tentative metabolic pathways of Bassa in rice plants and soils were suggested as shown in Fig. 8.

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要 約

殺虫剤、2-*sec*-butylphenyl *N*-methylcarbamate (バッサ®, BPMC) の水稻における吸収、移行と代謝、および土壤中における分解を、側鎖の *sec*-butyl 基の1位の methyl 基を ¹⁴C で標識した化合物を用いて検討した。水稻では、¹⁴C-バッサは根や葉面から吸収され、上部の生長中の部位や葉の先端に移行するがおもに、揮散により消失して、収穫時の残留は少なかった。稲体内に吸収されたバッサは代謝されておもに、*sec*-butyl 基、*N*-methyl 基および芳香環の4位が酸化を受け、生成物はさらに、β-グルコースまたは他の物質との抱合体を生じた。主代謝物は、2-(1-methyl 2-hydroxypropyl)phenyl *N*-methylcarbamate であった。土壤中では、分解物はおもに、2-(1-acetyethyl)phenyl *N*-methylcarbamate、2-*sec*-butylphenylcarbamate および 2-*sec*-butylphenol であった。ほかに土壤に強く結合される ¹⁴C が存在し、経時的に増加した。バッサの代謝物の生物学的評価をコリンエステラーゼの阻害度から検討した結果、代謝物の一つである 2-*sec*-butyl 4-hydroxyphenyl *N*-methylcarbamate はバッサよりも阻害が強かったが、この代謝物は水稻中では遊離形としては非常に少なく、ほとんどが抱合体として存在していた。そしておもなほかの代謝物はバッサよりも阻害が弱かった。

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